US-PAT-NO: 7118923
DOCUMENT-IDENTIFIER: US 7118923 B2
TITLE: **Nanoporous membrane** immunosensor

DATE-ISSUED: October 10, 2006

PRIOR-PUBLICATION-INFORMATION:

DOCUMENT-IDENTIFIER	DOCUMENT-DATE
US 20040126899 A1	July 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lee; Gil U.	West Lafayette	IN	N/A	US
Yanavich; Carolyn	Alexandria	VA	N/A	US

ASSIGNEE INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The United States of America as represented by the Secretary of the Navy	Washington	DC	N/A	US	06

APPL-NO: 10/734241
DATE FILED: December 15, 2003

CONTINUITY DATA:

division parent-doc US 09614727 00 20010712 US 6676904 A 20040113 child-doc US 10734241

INT-CL-ISSUED:

TYPE	**IPC DATE IPC-OLD**
IPCP	G01N33/543 20060101 G01N033/543

INT-CL-CURRENT:

TYPE	**IPC DATE**
CIPP	G01 N 33/543 20060101

US-CL-ISSUED: 436/518 , 436/524 , 436/164 , 436/172 , 435/6 , 435/7.1 , 435/287.1 , 435/422 , 435/101

US-CL-CURRENT: 436/518, 435/101 , 435/287.1 , 435/422 , 435/6 , 435/7.1 , 436/164 , 436/172 , 436/524

FIELD-OF-CLASSIFICATION-SEARCH: 435/6; 435/7.1 ; 435/287.1 ; 436/518 ; 436/524 ; 436/526 ; 436/164 ; 436/172 ; 422/57 ; 422/58 ; 422/101 ; 210/600 ; 210/321.75 ; 210/348

****See application file for complete search history****

REF-CITED:

U.S. PATENT DOCUMENTS

PAT-NO	**ISSUE-DATE**	**PATENTEE-NAME**	**US-CL**
4493815	January 1985	Fernwood et al.	N/A
4632901	December 1986	Valkirs et al.	N/A
4777021	October 1988	Wertz et al.	N/A
4797259	January 1989	Matkovich et al.	N/A
5100626	March 1992	Levin et al.	N/A
5137634	August 1992	Butler et al.	N/A
5137804	August 1992	Greene et al.	N/A
5830680	November 1998	Meyerhoff et al.	435/7.92
5843767	December 1998	Beattie et al.	N/A
6051380	April 2000	Sosnowski et al.	435/6
6180418	January 2001	Lee et al.	N/A
6225131	May 2001	van Damme et al.	436/524

6303389	October 2001	Levin et al.	N/A
6352862	March 2002	Davis et al.	N/A
6383748	May 2002	Carpay et al.	N/A
6692915	February 2004	Nallur	435/6

OTHER PUBLICATIONS Webster's II New Riverside University Dictionary, 1994, p. 875. cited by examiner

ART-UNIT: 1641
PRIMARY-EXAMINER: Le; Long V.
ASSISTANT-EXAMINER: Counts; Gary W.
ATTY-AGENT-FIRM: Ressing; Amy L. Karasek; John J.

ABSTRACT:

A sensor for a selected analyte in a test sample has (a) a semipermeable **membrane with pores** for retaining the analyte, where the **membrane** has been chemically modified by attachment of **membrane** modifiers; (b) immunoassay labels which have label binding ligands where these label binding ligands will have a binding affinity for the **membrane** modifiers in the presence of the analyte, and a measurably different binding affinity for the **membrane** modifiers in the absence of the analyte; and (c) a label detecting system, for detecting the presence of the labels on the **membrane**.

9 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

Abstract Text - ABTX (1):

A sensor for a selected analyte in a test sample has (a) a semipermeable **membrane with pores** for retaining the analyte, where the **membrane** has been chemically modified by attachment of **membrane** modifiers; (b) immunoassay labels which have label binding ligands where these label binding ligands will have a binding affinity for the

membrane modifiers in the presence of the analyte, and a measurably different binding affinity for the **membrane** modifiers in the absence of the analyte; and (c) a label detecting system, for detecting the presence of the labels on the **membrane**.

TITLE - TI (1):

Nanoporous membrane immunosensor

Brief Summary Text - BSTX (3):

The present invention relates generally to assays and more specifically to binding assays, such as antibody/hapten or DNA interactions, using **nanoporous membranes** for increasing the local concentration of the analyte, to improve the overall sensitivity of the assay. This technique can be used with a wide range of labeling schemes, including radio labeling and labeling with magnetic beads.

Brief Summary Text - BSTX (9):

Solid **supports** are used in many immunoassays, typically as adsorbent **layers**. Many of these, such as nylon and nitrocellulose **membranes have pore sizes** greater than 25 nm, to amplify the signal by increasing the surface area of the assay.

Brief Summary Text - BSTX (10):

Some microbiological assays use **membranes** to separate and concentrate bacteria. These **membranes** are typically on the order of 200 nm **pore size**.

Brief Summary Text - BSTX (11):

Viruses have been identified with aluminum ultrafiltration **membranes** with 20 nm **pores**. Organisms immobilized on these **membranes** have been identified using both specific and nonspecific dyes.

Brief Summary Text - BSTX (12):

Chemically selective **membranes** are used in some chemical sensors to pass the analyte through the **membrane**. Larger molecules are not allowed to pass through the **membrane** into the internal sensing solution.

Brief Summary Text - BSTX (18):

An aspect of the present invention is a sensor for a selected analyte in a test sample having (a) a semipermeable **membrane with pores** for retaining the analyte, where the **membrane** has been chemically modified by attachment of **membrane** modifiers; (b)

immunoassay labels which have label binding ligands where these label binding ligands will have a binding affinity for the **membrane** modifiers in the presence of the analyte, and a measurably different binding affinity for the **membrane** modifiers in the absence of the analyte; and (c) a label detecting system, for detecting the presence of the labels on the **membrane**.

Brief Summary Text - BSTX (19):

Another aspect of the invention is a method for detecting an analyte in a test sample, having the steps: (a) modifying a side of a semipermeable **membrane, the membrane having pores** for retaining the analyte, with **membrane** modifiers; (b) placing the test sample in contact with the **membrane** on the side of the **membrane with the membrane** modifiers; (c) drawing the test sample through the **membrane,** osmotically or with the application of differential pressure across the **membrane,** so that any analyte present in the test sample is drawn towards the modified **membrane** surface; (d) disposing immunoassay labels on the side of the **membrane with the membrane** modifiers, where these labels have label binding ligands where these label binding ligands will have a binding affinity for the **membrane** modifiers in the presence of the analyte, and a measurably different binding affinity for the **membrane** modifiers in the absence of the analyte; and (e) detecting the presence of the immunoassay labels on the **membrane**.

Description Paragraph - DETX (3):

FIG. 1 is a schematic representation of a **membrane** for a preferred embodiment of the invention.

Description Paragraph - DETX (10):

Binding members, such as the analytes and the **membrane** and label modifiers of the invention, refers to a member of a binding pair, i.e., two different molecules wherein one of the molecules specifically binds to the second molecule through chemical or physical means. In addition to the well-known antigen and antibody binding pair members, other binding pairs include, but are not intended to be limited to, biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, complementary peptide sequences, effector and receptor molecules, enzymes cofactors and enzymes, enzyme inhibitors and enzymes, a peptide sequence and an antibody specific for the sequence or the entire protein, polymeric acids and bases, dyes and protein binders, peptides and specific protein binders (e.g., ribonuclease, S-peptide and ribonuclease S-protein), sugar and boronic acid, and similar molecules having an affinity which permits their associations in a binding assay. Furthermore, binding pairs can include members that are analogs of the original binding member, e.g., an analyte-analog or binding member made by recombinant techniques or molecular engineering. If the binding member is an

immunoreactant it can be, e.g., an antibody, antigen, hapten, or complex thereof, and if an antibody is used, it can be a monoclonal or polyclonal antibody, a recombinant protein or antibody, a chimeric antibody, a mixture(s) or fragment(s) thereof, as well as a mixture of an antibody and other binding members. The details of the preparations of such antibodies, peptides and nucleotides and their suitability for use as binding members in a binding assay are well-known to those skilled-in-the-art.

Description Paragraph - DETX (12):

Preferably, the **pore** diameter of the **membranes** of the present invention is selected to retain the particular analyte of interest. Preferably, the **pore** diameter of the **membranes** of the present invention is selected to pass potential interferents that are smaller than the analyte of interest. For some embodiments, it will be preferred that the **pore** diameter does not exceed 25 nm. For other embodiments, it will be preferred that the **pore** diameter does not exceed 10 nm. This is sufficient to retain most analytes of interest. For larger analytes of interest, e.g. bacteria, it will be preferred to have **pore** diameters on the order of about 100 nm, to retain bacteria but to pass viruses. For even larger analytes of interest, e.g. pollen, spores, and dust particles, it will be preferred to have **pore** diameters on the order of about 1000 nm, to retain these particles but to pass bacteria and viruses.

Description Paragraph - DETX (13):

Referring to FIG. 1, in a preferred embodiment of the invention, a semipermeable **membrane** 10 has **membrane** modifiers 12 (typically antibodies) bound to the **membrane** through linkers 14. These linkers can be chosen from a wide range of linkers used in surface chemistry, but typically will be hydrophillic polymer films. The **pores** 16 preferably have nominal diameters less than 25 nm.

Description Paragraph - DETX (14):

The **membrane** modifiers may be the same across the surface of the **membrane,** or several different types of **membrane** modifiers may be patterned in an array, to allow for parallel processing within a single test vessel.

Description Paragraph - DETX (15):

Referring to FIG. 2, this **membrane** may be used to rapidly transport the analyte 18 to the binders if a differential or osmotic pressure is applied across the **membrane**. This pressure, from a pressure source (not shown) such as a pump, causes bulk flow from a volume on one side of the **membrane** 48 to a volume on the other side of the **membrane** 50, as indicated by the dashed arrows. If a differential pressure source is used (as distinct from osmotic pressure), the pressure may be positive or negative. That is, the pump may be configured to increase the pressure on the side of the **membrane** with the test sample,

or to decrease the pressure on the distal side of the **membrane**. A labeled binder 20 is then used to sense the binding event using a detector (not shown in this figure).

Description Paragraph - DETX (16):

The labeled binders 20 may be the same, or several different types of labeled binders may be patterned used, typically in conjunction with a **membrane** patterned with several different hmembrane modifiers in an array, to allow for parallel processing within a single test vessel.

Description Paragraph - DETX (18):

Ultrafiltration **Membranes** for Sensing

Description Paragraph - DETX (19):

The rate of a chemical reaction at a surface such as a **membrane** is controlled by at least three phenomena. First, the reactant must be transported to a surface, which is a process that can be controlled by diffusion or convection. If pressure is applied across the **membrane,** mass transport will be dominated by the convective flow of the sample towards the **membrane**. The second phenomenon, controlling the overall reaction rate, is the kinetics of the reaction at the interface. In the case of an antibody-antigen interaction, this reaction can be written B+A.revreaction.B-A where B is the binder, A is the analyte and B-A is the binder-analyte complex. The amount of bound analyte is determined by the equilibrium constant of the reaction K.sub.eq=k.sub.1/k.sub.-1=[B-A]/[B][A] where k.sub.1 and k.sub.-1 are the forward and reverse rate constants, respectively. The third phenomenon controlling the rate of reaction is the diffusion of the analyte away from the surface. The advantage of using **membranes is that if the membrane** retains a significant portion of an analyte, the analyte will be rapidly concentrated at the **membrane** surface resulting in an increase in the amount of analyte bound. Conventional **membrane**-based immunoassays bind the analyte in their micron **size pores** producing an active area 1 100 microns deep in the **membrane**. The ultrafiltration **membrane in this invention has a pore size** of 25 nm or less, which acts to concentrate the analyte at the surface of the **membrane**. The observed increase in sensitivity of the assay executed on the ultrafiltration **membranes** in this invention is attributed to the higher concentration of analyte in the active area of the detector.

Description Paragraph - DETX (20):

Ultrafiltration **membranes** are widely used for separation and purification processes. The **pore size of the membrane,** the filtration pressure and the physical properties of the species determine the efficiency of retention of a species. Many of these **membranes** are composed of organic polymeric materials. Nucleopore **membranes,** an example of a

polymer ultrafiltration **membrane,** are composed of polycarbonate, have **pore sizes** of 10 10,000 nm and **pore densities** of approximately 10.sup.12 m.sup.-2 (3). Several inorganic ultrafiltration **membranes** are also available: (i) Anopore.RTM. (**membranes** which are anodically etched from aluminum, have **pore sizes** ranging from 10 250 nm, and **pore density** of 10.sup.12 10.sup.15 m.sup.-2 (4); (ii) Nanochannel glass **membranes** are drawn from optical fibers and have similar properties to the Anopore **membrane**; (iii) Microfabricated **membranes** currently have larger **pore sizes** and low **density of pores** than the Anopore **membranes**.

Description Paragraph - DETX (21):

For this invention, it is desirable to have an ultrafiltration **membrane** that has a high **density of pores** to avoid the need for high differential pressures. In addition, for applications with optical detection it is important that the **membrane** be flat, optically translucent and not likely to change shape or **size** during the course of the assay. Organic ultrafiltration **membranes** do not meet these requirements. The inorganic **membranes** have the physical properties required for this assay: (i) high flow rates with moderate pressures; (ii) optically translucent **membranes** that retain their shape when wet; (iii) high retention efficiencies for macromolecules. Studies of 10 nm **pore size** Anopore **membranes** at pressures of 100 kPa or less indicate that 6, 20 and 66 percent of 30,000, 67,000 and 150,000 Da proteins are retained, respectively.

Description Paragraph - DETX (22):

Methods of Preparing Activated **Membranes**

Description Paragraph - DETX (23):

The ultrafiltration **membrane** in this invention must be functionalized with a binder in order to act as a sensor. However, fouling is a phenomenon that will severely limit the use of ultrafiltration **membranes** for sensing. Protein fouling has been attributed to adsorption, **pore** plugging and cake consolidation. We have minimized the effect of protein fouling by executing the immunoassay on a dense hydrophilic polymer film that inhibits protein adsorption. In a preferred embodiment, the active surface of an Anopore **membrane** was coated with a dense **layer** of biotinylated poly (ethylene glycol) (PEG) using a polyethylene imine (PEI) adhesion **layer** (see Example 1). Many variations on this chemistry can be used: (i) The surface can be activated using several different approaches, e.g., silanization or thiolation. (ii) Other hydrophilic polymers could be used to inhibit protein adsorption, e.g., dextran. (iii) Other approaches to inhibit nonspecific protein adsorption are available, e.g., adsorption of proteins such as bovine serum albumin (BSA). The biotin-PEG functionalized **membranes** have the following advantages: (i) the **membranes** can be stored in a dry form for extended periods of time; (ii) the concentration of receptors on the **membrane** can be varied; (iii) the surface can

be regenerated. It is especially desirably to make these **membranes** reusable due to their cost.

Description Paragraph - DETX (24):

Many types of binders can be immobilized on the **membrane**. Binders against specific analytes may be either directly or indirectly bound to a hydrophilic polymer film. Preferred binders include DNA oligonucleotides, PNA oligonucleotides, polyclonal antibodies and monoclonal antibodies. In a preferred embodiment, antibodies are specifically immobilized on the biotinylated PEG surface using antibody-streptavidin conjugates (see Example 3). Alternatively, binders may be directly bound to the hydrophilic polymeric films functionalized with N-hydroxysuccinimide (NHS), maleimide, or vinyl groups. For example, antibodies can be thiolated with N-succinimidyl S-acetylthioacetate and then reacted with .alpha.-vinyl sulfone, .omega.-n-hydroxysuccinimide PEG, 3,000 MW functionalized polymers. There are of course many variations on this chemistry.

Description Paragraph - DETX (26):

The assay method will be composed of at least three steps: (i) preparation of the sample, (ii) reaction of the analyte at the **membrane** surface and (iii) detection of the binding events.

Description Paragraph - DETX (29):

TABLE-US-00001 Scheme 1: Direct Sandwich Capture **membrane** Complex **Membrane**-binder analyte - binder-label **Membrane**-biotin streptavidin-binder - analyte - binder-label

Description Paragraph - DETX (30):

This assay can be executed in a series of sequential steps on the **membrane** or the complex could be formed in solution and then bound on the **membrane**. Note that convective transport may be used at each step of the assay to enhance the response time.

Description Paragraph - DETX (31):

TABLE-US-00002 Scheme 2: Indirect Sandwich Capture **membrane** Complex **Membrane**-binder analyte - binder - antibinder-binder-label **Membrane**-biotin streptavidin-binder - analyte - antibinder-binder-label

Description Paragraph - DETX (32):

In this case, it would probably be most convenient to execute the assay on the **membrane**. Again, convective transport may be used at each step of the assay to enhance the response time.

Description Paragraph - DETX (37):

The assay apparatus will be composed of a **membrane,** fluid handling system, and detector. A fluid handling system and detector are described in this section.

Description Paragraph - DETX (38):

The differential pressure necessary to transport the analyte to the **membrane** surface may be produced osmotically, or with either positive pressure or negative pressure from a pump. In cases in which the sample will be handled batch wise conventional vacuum filtration equipment or a syringe filtration apparatus may be used (see examples).

Description Paragraph - DETX (39):

Biosensors typically require automated processing and continuous operation. A schematic of a cell for performing continuous assays is shown in FIG. 3. This 1.times.0.25.times.0.5" 21 cell is drawn with five reaction chambers 22, 24, 26, 28, 30 in which the sample passes from left to right. This cell makes it possible to run five sequential assays if each surface used one time. If the surfaces can be regenerated, numerous assays can be run in a single cell. In this cell the active area of each chamber is patterned with four binders 32, 34, 36, 38. Patterning a surface with binders has the advantage that multiple analytes can be detected while simultaneously running internal standards. Techniques for patterning binders on solid surface are well known to those skilled in the art. A preferred embodiment would be to use ink jet technology to accurately distribute small volumes of binder-streptavidin conjugates on specific areas of biotin-PEG functionalized **membrane** 10. This cell includes a manifold 40 that will allow the sample to be channeled to the active area of the **membrane** and pass through the cell. Clearly, this is not the only geometry in which this cell could be constructed.

Description Paragraph - DETX (45):

The choice of dye may be varied widely, being primarily chosen to provide an intense color with minimum absorption by the immunosorbing zone **support**. Illustrative dye types include quinoline dyes, triarylmethane dyes, acridine dyes, alizarin dyes; phthaleins, insect dyes, azo dyes, anthraquinoid dyes, cyanine dyes, phenazathionium dyes, and phenazoxonium dyes.

Description Paragraph - DETX (66):

Preferably, the force differentiation assay, described in co-pending application Ser. No. 09/008,782, is used. In this technique, the **membrane** and the magnetic beads are modified with specific binding agents, so that these bead modifiers will have a binding affinity for the **membrane** modifiers in the presence of the analyte species, and a measurably different binding affinity for the **membrane** modifiers in the absence of the analyte species. The beads and the test sample are introduced into the test vessel, and an adjustable magnetic field source is used to apply a magnetic force to the beads. An imaging system is used to determine whether the beads are bound to the **membrane,** thereby testing for the analyte.

Description Paragraph - DETX (67):

Referring to FIG. 4, depicting a sandwich assay configuration for the present invention, a **nanoporous membrane** 10 is disposed in a test vessel, dividing the test vessel into two volumes 48, 50. Magnetic beads 19 are disposed in the vessel, within the first volume 48.

Description Paragraph - DETX (68):

The beads 19 are modified with molecules which are referred to herein as bead modifiers 23, and the **membrane** 10 is modified on the side facing the first volume with **membrane** modifiers 12. Both of these types of modifiers will be selected from those molecules that are capable of recognizing and selectively binding other molecules, including antibodies, haptens, polynucleic acids, polypeptides, glycolipids, hormones, polymers, metal ions, and certain low molecular weight organic species (see above).

Description Paragraph - DETX (69):

The mechanism for applying a variable, normal magnetic field to the beads 19 is shown here as a movable annular magnet 42. The annular magnet produces a uniform [right arrow over (B)] field which is oriented along its axis across millimeter **size** areas. It has been discovered that a millimeter scale NdFeB magnet can apply a uniform field over sample areas consistent with imaging by optical microscopy, if the magnet is carefully centered. This normal B field acts upon the bead 10 to create a normal force (F) on the beads 19, which in turn puts tension on the bond between the bead 19 and the **membrane** 10. Since covalent bonds are typically much stronger than specific molecular interactions (such as antibody-hapten interactions), the strength of the linkage between the bead and the **membrane** is limited by the strength of this specific molecular interaction.

Description Paragraph - DETX (74):

Additionally, the imaging system for the present invention preferably has the capacity to discriminate between single beads on the substrate and clusters of two or more beads on the substrate, based on their **size**. It has been discovered that non-uniform surface

chemistries and magnetic fields of the structures taught by Rohr produce the following non-ideal behavior.

Description Paragraph - DETX (77):

Nonspecific adhesion between beads, and between beads and the **membrane,** appears to increase when the beads are loaded with proteins, which suggests protein-protein interactions are the primary source for this adhesion.

Description Paragraph - DETX (81):

Surface modification chemistries (described below) have been developed that consistently produce very low nonspecific adhesive forces in a majority of beads. Typically, 80 98% of the beads can be removed from a surface at force equivalent to their buoyant weight, i.e., .apprxeq.40 femtoNewtons (fN) in the case of Dynal's M280 beads. The number of specific molecular interactions linking a bead to the surface will depend on the **density** and flexibility of ligands and receptors on the bead and surface.

Description Paragraph - DETX (85):

Preparation of Functionalized **Membrane**: PEG Biotin **Membrane** with Streptavidin-Antibody Conjugate.

Description Paragraph - DETX (86):

Anodisc **membrane** was hydrated with 200 ml of pure water for 1 minute. Excess water was removed, and the **membrane** was rinsed with 50 mM sodium bicarbonate buffer (NaHCO.sub.3) pH 8.2. Excess buffer was removed from themembrane and 5% (w/v) PEI in 50 mM sodium bicarbonate buffer (NaHCO.sub.3) pH 8.2 was added. Incubation was at room temperature for 1 hour.

Description Paragraph - DETX (87):

The **membrane** was rinsed three times with water and once with 50 mM sodium bicarbonate buffer (NaHCO.sub.3) pH 8.2. Excess liquid was removed from the **membrane,** followed by the addition of a-biotin, w-NHS poly(ethylene glycol)-carbonate, MW 3,400 (Shearwater Polymers, Huntsville, AL) at 20 mg/mL in 50 mM sodium bicarbonate buffer (NaHCO.sub.3) pH 8.2. The PEG was incubated at room temperature for 2 hours, excess PEG-biotin solution was washed off with three rinses of water and stored dry.

Description Paragraph - DETX (88):

A sandwich immunoassay was built on the PEG-biotin functionalized Anodisc **membranes**. The **membranes** were rehydrated with 200 ml of water for 1 minute and rinsed with 0.1 M phosphate buffered saline (PBS) pH 7.4. Next 1% (w/v) bovine serum albumin (BSA) (Sigma Chemical Company, St. Louis, Mo.) was added to the surface for 1 hour to block non-specific sites. The **membrane** was then washed 3 times using PBS with 0.05% Tween 20 (PBST). Next, the appropriate antibody-streptavidin conjugates were diluted in PBST and added to the **membrane** in 200 ml volumes. The optimal conjugate concentration was determined by serial dilution analysis. Rabbit antibody-streptavidin for B. globigii and Goat antibody-streptavidin for ovalbumin and MS-2 were used as the capture antibodies. The conjugates were incubated on the surface for 1 hour, then excess antibodies were removed by washing 3 times using PBST and rinsing once with PBS. The surfaces can be stored in PBS at 4 C for up to 12 hrs.

Description Paragraph - DETX (91):

A functionalized **membrane** was incubated with the appropriate dilution of goat antibody-(ovalbumin)-streptavidin conjugate (200 ml) and then washed with PBST to remove unbound antibodies as described in Example 1.

Description Paragraph - DETX (92):

The **membrane** was then placed into a glass microanalysis vacuum **membrane** holder (Fisher Scientific, Springfield, N.J.) composed of a borosilicate glass funnel, base, fritted glass **support,** spring clamp, and No. 5 stopper. A 1 mL sample of a standard solution of known concentration (analytes=Ova ng/ml=0, 10, 1, 0.1, 0.01) was added to the filtration holder and incubated for 5 10 minutes. The analyte solution was then filtered using a water aspirator for 5 minutes. Unbound analyte was removed by washing the **membrane** 3 times with PBST. A sandwiching antibody, rabbit-antibody-ovalbumin (affinity purified), was then added to the **membrane** (200 .mu.l) at 2 .mu.g/ml in PBST and incubated for 1 hour. The more ovalbumin present in the test solution the more 2.sup.nd antibody will bind. The **membrane** was then washed three times with PBST, and rinsed with PBS. The **membrane** was then placed onto a glass microscope slide, excess fluid was removed, and anti-rabbit IgG-Seramag beads diluted in 0.1% (w/v) BSA were added to the **membrane** and incubated for 30 minutes.

Description Paragraph - DETX (93):

The samples were analyzed using a Carl Zeiss Axiovert 100 TV inverted microscope fitted with a motorized stage (Ludl Electronics, N.Y.). A custom written software program operated the reader, this enabled computer control of hardware via a serial port. The image analysis was performed in real time by accessing routines provided by Image Pro plus, version 3.0 commercial imaging software (Media Cybernetics). The **membrane** was analyzed in 3 positions consisting of a 128.times.96 mm area at a distance of 200

.mu.m apart using a water immersion objective (63.times.), 0.9 numerical aperture (NA) (Carl Zeiss) before and after exposure to the magnet. A magnetic force, was applied using a NdFeB block magnet (Magnet Sales and Mfg. Co., Culvert City, Calif.) magnetized perpendicular to the substrate. The magnet was placed 0.1 mm from the surface for 10 seconds to remove unbound beads from the sample surface. The remaining beads were then counted.

Description Paragraph - DETX (97):

A functionalized **membrane** was incubated with the capture antibody, goat antibody-(MS-2)-streptavidin conjugate, and rinsed with PBST as stated in Example 1. Follow the same procedure as stated in Example 2 with these few exceptions: capture antibody used was goat antibody-(MS-2)-streptavidin conjugate; analytes=MS-2=0, 10.sup.5, 10.sup.4, 10.sup.3 pfu/ml; sandwiching antibody, Rabbit-antibody-MS-2, was then added to the **membrane** (200 .mu.l) at 1 .mu.g/ml in PBST. The average binding for the various analytes are as follows (pfu MS2): 0=4%, 10.sup.5=66.5%, 10.sup.4=35.4%, 10.sup.3=22% The results show a sensitivity for MS-2 exceeding 10.sup.3 pfu/ml.

Description Paragraph - DETX (101):

A functionalized **membrane** was incubated with Rabbit antibody-(BG)-streptavidin conjugate and rinsed with PBST. In order to break apart B. globigii, the sample was first subjected to bead mill homogenization (18). Glass beads (0.1 1 mm) were added to a 0.75 ml BG solution: 1 g glass beads per microcentrifuge tube. The sample was added to the bead beater (Mini-Bead Beater-8, Bio-Spec products) and mixed on "Homogenize" for 3 minutes. The samples were then centrifuged at 12,000.times.g for 6 minutes. The supernatant was then removed and recentrifuged for 2 minutes at 12,000.times.g to eliminate any extraneous glass particles.

Description Paragraph - DETX (103):

1 ml of supernatant was removed and added to the filtration apparatus for incubation with the functionalized surface (analytes=BG=0, 10.sup.5, 10.sup.4, 10.sup.3, 10.sup.2.5 cfu/ml) for 5 10 minutes and then filtered using a water aspirator for 5 minutes. Unbound analyte was removed by washing the **membrane** 3 times with PBST, then a sandwiching antibody, goat-antibody--B. globigii, was added to the **membrane** (200 .mu.l) at 2 .mu.g/ml in PBST and incubated for 1 hour. The more B. globigii present in the test solution the more 2.sup.nd antibody will bind. The **membrane** was then washed three times with PBST, and rinsed with PBS.

Description Paragraph - DETX (104):

The **membrane** was then placed on a glass microscope slide, excess fluid was removed, and anti-goat IgG-Seramag beads diluted in 0.1% (w/v) BSA were added to the **membrane** and incubated for 30 minutes. The analysis of the samples was the same as in Example 2. The average binding for the various analytes are as follows (cfu B. globigii): 0=14%, 10.sup.5=93%, 10.sup.4=85%, 10.sup.3=66%, 10.sup.2.5=44%. The sensitivity for B. globigii exceeding 10.sup.2.5 cfu/ml

Description Paragraph - DETX (107):

A functionalized **membrane** was incubated with goat antibody--(ovalbumin)--streptavidin conjugate and rinsed with PBST as described in Example 1. The same procedure as stated in example 2 was followed with these few exceptions: Capture antibody was goat antibody-(ovalbumin)-streptavidin conjugate; analytes=Ova ng/ml=0, 10, 1, 0.1, 0.01, Ova 1 ng/ml+MS-2 10.sup.8 pfu/ml, Ova 1 ng/ml+MS-2 10.sup.5 pfu/ml, Ova 0.01 ng/ml+MS-2 10.sup.8 pfu/ml, Ova 0.01 ng/ml+MS-2 10.sup.8 pfu/ml; the sandwiching antibody, rabbit-antibody-ovalbumin (affinity purified), added was at 2 .mu.g/ml in PBST. The **membrane** was then washed three times with PBST, and rinsed with PBS.

Description Paragraph - DETX (108):

The **membrane** was then placed on a glass microscope slide, excess fluid was removed, and anti-rabbit IgG-Seramag beads diluted in 0.1% (w/v) BSA were added to the **membrane** and incubated for 30 minutes. The results show a sensitivity of 0.01 ng for Ovalbumin. The total binding decreases by 32% in the Ova 1 ng/ml+MS-2 10.sup.8 pfu/ml and the remainder of the mixed analyte assays show a decrease of 10% in binding. This is most likely caused by steric hindrance.

Description Paragraph - DETX (111):

A functionalized **membrane** was incubated with goat antibody--(ovalbumin)--streptavidin conjugate and rinsed with PBST as described in example 1. The **membrane** was then placed into the glass microfilter holder and a 1 mL sample (analytes=Ova ng/ml=0, 10, 1, 0.1, 0.01) was added to the filtration holder and incubated for 5 10 minutes. The analyte solution was then filtered using a water aspirator for 5 minutes. Immediately following filtration of the analyte, the sandwiching antibody, rabbit-antibody-ovalbumin (affinity purified), was then added to the **membrane** at 1 .mu.g (0.5 .mu.g/ml over a 2 ml volume). The secondary antibody was passed through the **membrane** slowly to concentrate the antibody at the interface. The **membrane** was then washed three times with PBST, and rinsed with PBS. The **membrane** was then placed on a glass microscope slide, excess fluid was removed, and anti-rabbit IgG-Seramag beads diluted in 0.1% (w/v) BSA were added to the **membrane** and incubated for 30 minutes. The **membrane** was analyzed as described in Example 2. The results were as follows (ng

Ovalbumin): 0=15%; 1=81%; 0.01=55%. The sensitivity of the Ovalbumin assay with filtration of 2.sup.nd antibody was 0.01 ng.

Claims Text - CLTX (1):

1. A method for detecting a selected analyte in test solution, comprising providing a sensor comprising: a test vessel; a semipermeable **membrane having pores** for retaining the analyte, wherein said semipermeable **membrane** divides said test vessel into a first volume and a second volume, wherein said **pores** are selected to prevent the analyte from passing into or through said **membrane, wherein said membrane** is chemically modified by attachment of **membrane** surface modifiers on at least a side facing said first volume but not within said **pores, wherein said membrane supports** a 100 kPa pressure load, said **membrane** is functionalized with a binder at the surface of said **membrane** in order for said **membrane** to act as a sensor; assay labels, disposed within said first volume, said assay labels having label modifiers, said label modifiers having a binding affinity for the **membrane** modifiers in the presence of the analyte, and a measurably different binding affinity for said **membrane** modifiers in the absence of analyte; a pressure source, for driving said test solution from said first volume into said second volume through said modified surface of said **membrane** contacting the test solution with said **membrane** and; removing labels not bound to said analyte and; detecting the presence or absence of said analyte by detecting the presence or absence of said labels on said **membrane**.

Claims Text - CLTX (2):

2. The method of claim 1 wherein said **membrane has pores** not greater than 10 nm in diameter.

Claims Text - CLTX (3):

3. The method of claim 1, wherein said **membrane has a pore density** of at least 10.sup.15/m.sup.2.

Claims Text - CLTX (4):

4. The method of claim 1, wherein said **membrane** is essentially flat and optically translucent when wet.

Claims Text - CLTX (5):

5. The method of claim 4, wherein said **membrane** remains translucent, and the shape of said **membrane** remains flat even under pressure associated with flow of solution through said **membrane**.

Claims Text - CLTX (6):

6. The method of claim 1, wherein said **membrane** is an aluminum oxide **membrane**.

Claims Text - CLTX (7):

7. The method of claim 1, wherein said modified side of said **membrane** is modified with a biotin-polyethylene-glycol (PEG) using a polyethyleneimine (PEI) **layer**.

Claims Text - CLTX (8):

8. The method of claim 1 wherein said **pores** allow a solvent to pass through while preventing flow of said binder, analyte, or assay labels.

Claims Text - CLTX (9):

9. The method of claim 1, wherein said **membrane has pores** not greater than 20 nm in diameter.